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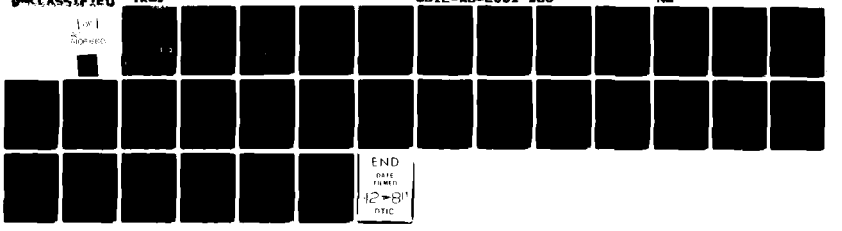

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The ability of personnel to operate in environments where they may be exposed to new infectious agents may well be dependent on their genetic ability to mount a successful immune response to the disease. The response could be to an active infection causing disease or to a protective vaccine. The immune response to a variety of foreign agents (antigens) is handled by the immune system in an essentially similar manner whether the agents be virus, bacteria, parasite, transplanted tissue or vaccine. One of the major

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advances in the field of biology over the past 15 years has been the discovery that the response to various infectious agents in some mammals is under the control of inherited genes many of which reside within the major histocompatibility complex (MHC). Therefore, study of the MHC in man in relationship to infectious disease may identify the genetic factors involved in resistance or susceptibility to infectious agents. Such information would then allow one to predict the capability of an individual to respond to a given infectious agent or vaccine.

The patient population chosen for the index study comprised individuals who during the newborn period suffered respiratory syncytial virus (RSV) infection, where a small number developed severe bronchiolitis requiring hospitalization while most recovered with no ill effects. Families of patients with RSV disease who were willing to participate were identified, blood samples obtained and the lymphocytes separated and cryopreserved. A total of 63 patients and their families were HLA-A, B, C typed. In addition, the patients were HLA-DR typed. No significant association at the p 0.01 level was observed for any HLA antigen. The number of mismatched haplotypes between patient and siblings was determined. The distribution of mismatched haplotypes between RSV patients and unaffected siblings was not different from that expected under the null hypothesis of no linkage. However, the distribution of mismatched haplotypes between RSV patients and siblings who had also been hospitalized with lower respiratory disease was different from that expected under the null hypothesis of no linkage. In addition, a significant distortion (p 0.01) in the inheritance of the maternal haplotype was observed. The number of families with more than 1 sibling hospitalized with lower respiratory tract disease is small (six) and needs to be enlarged. The working hypotheses are (a) the presence of a high responder gene(s) that confers resistance to disease in adult life but lead to severe complications to viral infection in the newborn or (b) a lethal haplotype. Both hypotheses are testable. In summary, these studies suggest that certain genes in the HLA region may influence not only ability to respond to an infection but also certain harmful sequela.

Office of Naval Research

Contract N000-14-79-C-0886

Task No. NR 204-098

Technical Report. No. 2

Histocompatibility Typing for the Prediction Of Susceptibility To Infectious Disease

By

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and the

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OCTOBER 1981

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A. ABSTRACT

The objective of this contract is to determine if susceptibility to certain infectious agents or morbidity associated with infection can be predicted based on knowledge of HLA type. The contract was established to design and conduct studies that will indicate if one can predict an individual's ability to mount an immune response to an active infection causing disease or to a practical vaccine based on a knowledge of genes within the HLA region. The diseases, or complications under study are those selected by the project officer for investigation. In general the format of these studies will follow previously established guidelines which include:

a. careful disease classification or subclassification based on firm clinical and laboratory parameters

b. preparation of data collection forms based on items to be included in the analysis

c. collection and storage of cells

d. HLA typing of sample population (random populations exhibiting trait and/or families)

e. statistical analysis for association or linkage of HLA and disease parameter.

The population chosen for the index study comprises individuals who as infants suffered respiratory syncytial virus (RSV) infection, where a small number developed bronchiolitis requiring hospitalization while most recovered with no severe side effects. Association and/or linkage between HLA, virus infection (seroconversion) and morbidity (bronchiolitis) as well as a number of other factors has been evaluated within the random sample population and within families using standard statistical methods.

In addition, two other diseases were studied to determine if there was a component influencing susceptibility that was linked to the HLA complex. These diseases were an unusual form of cutaneous leishmania and chronic granulomatous disease.

B. INTRODUCTION

The following technical report summarizes the progress on ONR Contract N000-14-79-C-0886 "Histocompatibility Typing for the Prediction of Susceptibility to Infectious Disease" during the second contract year 10-1-80 to 9-30-81. This project was initiated in August, 1979, to determine whether the histocompatibility type of an individual could predict his capacity to respond to a given infectious agent or protective vaccine in such a manner as to provide him with resistance for the particular disease process, and to determine if susceptibility to morbidity associated with certain infections is related to HLA type.

During the first contract year a computer form was custom designed to be used as a questionnaire to assure completeness of data collection and the clinical and laboratory parameters to be analyzed were decided upon. Families of patients with RSV disease who were willing to participate were identified. Blood samples were collected, the lymphocytes separated and cryopreserved from 63 families compromising 262 individuals. During this time the HLA laboratory at Georgetown was expanded so as to have the capability of typing for HLA-A,B,C,DR, MB and MT locus alleles at a reference laboratory quality. The facility developed the capability of typing for all World Health Organization Nomenclature Committee approved specificities in addition to a number of new specificities which have not yet been given a W.H.O. designation.

During the present contract year, the 63 families comprising 262 individuals have been typed for HLA-A,B,C and the 63 patients also typed for HLA-DR. The results have been analyzed for association between disease and any given HLA specificity and also for genetic linkage to the major histocompatibility complex.

C. BACKGROUND

The ability of individuals to operate in environments where they may be exposed to new infectious agents may well depend on their genetic ability to mount a successful immune response and not only resist the disease but perhaps more importantly not to be susceptible to the more serious pathological consequences of an infection. The responses could be to an active infection or to a protective vaccine. The protective immune response to a variety of foreign agents (antigens) is handled by the immune system in an essentially similar manner whether the agent be virus, bacteria, parasitic, or transplanted tissue.

There is evidence to suggest that responses to infectious agents are under the control of genes in the major histocompatibility complex (MHC) of mammals. Some form of major histocompatibility complex appears to be present in all mammalian (and possibly chordate) species (1), thus, the MHC may play a vital functional role(s) which has allowed it to be conserved as a complex or "sugergene" throughout evolution. The MHC in humans includes a series of structural and regulatory genes. Among these are the structural genes for HLA-A,B,C (class I) alloantigens (i.e. those made up of glycoprotein with attached $_2$ subunit), and at least two (2-5) and probably a minimum of three (6,7) B cell specific (class II) alloantigens (i.e. those made up of two

glycoprotein chains) and, at least two types of T cell subpopulation alloantigens (8,9). In addition, there are lymphocyte activating determinants controlling stimulation in mixed lymphocyte culture (10), stimulation of primed lymphocytes (11), and stimulation of cytotoxic T cells (12). Genes controlling the production of various complement components (C2, C4, Bf), that include the components which are pivotal control points in both the classical and alternate pathways of complement activation (13,14) are also found in the HLA region. Also, the response to various antigens in those mammals studied is under the control of inherited genes many of which reside within the MHC (15,16). These genes have been termed "Immune response" or Ir genes. Although the presence of immune response genes is established in several mammalian species, there is only limited evidence for such genes in man. The evidence to suggest that humans have Ir genes comes from studies primarily in the area of responses to allergens, such as hayfever where it has been shown that the ability to become allergic is linked to genes within the human MHC (17).

We proposed to investigate in well controlled infectious disease models, whether in man the MHC genes contribute significantly to resistance or morbidity associated with infectious disease. These studies are intended to evaluate a number of disease systems.

Initially we chose to study a group of patients, all of whom suffered from respiratory syncitial virus (RSV) during the newborn period. This virus causes an upper respiratory infection and in some infants causes a serious complication with dyspnea and severe respiratory distress termed bronchiolitis which frequently is of such severity to merit hospitalization (18). The intent of the study was to determine whether there is a relationship (association or linkage) between HLA and those individuals who had severe consequences during

the infection period. The study of this disease serves as a well controlled infectious disease model in which linkage between HLA susceptibility and disease may be evaluated. It is meant to serve as a model for the study of other infectious diseases.

Two other diseases were also investigated: (1) a unique form of cutaneous Leishmania and (2) chronic granulomatous disease. HLA typing was performed to determine if there was a genetic component involved in susceptibility and to aid in design of studies investigating the cellular and functional aspects of the disease.

D. RESEARCH DESIGN AND PLAN

1. RESPIRATORY SYNCYTIAL VIRUS DISEASE PROTOCOL:

1.1 Establishment of guidelines for use in designing and conducting HLA disease association studies, and development of a computer data information sheet in the form of a questionnaire, to be used as a model for these studies. Such a questionnaire would include (a) all personal information such as age, sex and family relationships, (b) pertinent clinical evaluations and laboratory studies (virus culture, antibody titer, etc.) necessary for adequate diagnosis and classification of the disease and (c) HLA typing data.

1.2 Establishment of a contract facility to perform histocompatibility typing for HLA-A, B, C, DR and other B cell antigens (for example, the recently described MB and MT antigens) at a reference research laboratory level.

1.3 Identification of patients and families willing to participate in the study. In the first of these projects, the patients would be those who suffered from the severe complication of respiratory syncitial virus (RSV) requiring hospitalization during the newborn period and their families (both parents plus at least one unaffected sibling).

1.4 Collection and cryopreservation of lymphocytes from the above patients and family members.

1.5 HLA-A, B, C, DR and D typing (when appropriate) of the cryopreserved lymphocytes from the above patients and family members.

1.6 Analysis of the association and/or linkage if any between the HLA complex and disease susceptibility to RSV disease and its complications.

All aspects of the study have been completed to date. The manuscript is in preparation (copy attached).

2. DISSEMINATED CUTANEOUS LEISHMANIASIS (ORIENTAL SORE) PROTOCOL

2.1 Collect blood samples in the Dominican Republic and air ship to Washington, D.C. College blood samples at the airport in Washington, D.C., separate and cryopreserve the lymphocytes for later use.

2.2 HLA-A,B,C type the cryopreserved lymphocytes.

2.3 Analyze association and/or linkage to the HLA complex.

3. CHRONIC GRANULOMATOUS DISEASE PROTOCOL

3.1 Separate and cryopreserve lymphocytes from whole blood drawn from family members for later use.

3.2 HLA-A,B,C type the cryopreserved lymphocytes.

3.3 Analyze the data for segregation with the HLA complex.

E. RESULTS

1. RSV DISEASE STUDY

Items 1.1 through 1.4 under research plans were accomplished during the first contract year and reported in technical report no. 1.

1.5 HLA Genotyping

1.5.1 HLA A,B,C Typing. HLA typing for the HLA-A,B,C, allelic series has been accomplished in 63 families (27 Caucasian; 36 Black) including 262 individuals. The Amos modification of the N.I.H. typing technique which employs a wash step prior to the addition of complement was used (19). All World Health Organization (W.H.O.) Nomenclature Committee approved HLA and workshop specificities could be detected with the set of alloantisera used. In addition several HLA-A and B specificities (A19V, BU, SV, BF) could also be detected.

1.5.2 HLA-DR Typing. HLA phenotyping for HLA-DR as well as MB1,2,3 and MT1,2 has been accomplished for the 63 patients using the 2 color-fluorescence technique (20). This technique allows for HLA-DR typing using very small quantities of blood. This is particularly important in studies, such as this one, where children are involved. All W.H.O. nomenclature committee approved HLA and workshop specificities except DRw10 as well as the additional B cell antigens MB (3) and MT (21) were able to be defined with the set of alloantisera use.

1.6 Analysis of the HLA complex and disease susceptibility to RSV disease and its complications.

1.6.1 Association Study

A total of 63 patients (27 Caucasian; 36 Black) were HLA-A,B,C typed for 74 HLA antigens and DR typed for DR1-w9 plus MB1,2,3 and MT1,2. No significant association at the p less than 0.01 level was observed for any HLA antigen. The control frequencies used were those obtained during the 8th International Histocompatibility Workshop (22). Since HLA antigen frequencies for Caucasians from geographically different areas in North America have been shown to not differ statistically, the workshop antigen frequencies provide adequate control information for North American Caucasians. Similar comparisons of antigen frequencies for North American Blacks in different geographic areas have not been made. Therefore, the results for the Black patient group are preliminary. A local Black population is needed to make valid comparisons. The results are given in Tables I-IV.

1.6.2 Analysis of Family Data

Genotyping was accomplished by observation of segregation and haplotypes were assigned based on segregation. Traditionally, the father's haplotypes are always designated a,b and the mother's haplotypes are designated c,d. Thus, there are four possible combinations for offspring: ac, ad, bc, bd. The patients and their unaffected siblings haplotypes were tallied and are given in Tables V and VI. Families in which data was available for both parents (a/b x c/d) was analyzed separately from those in which the father was not available for typing (a/? x c/d).

1.6.2.1 RSV patient compared to unaffected siblings

The number of mismatched haplotypes between the patient and the unaffected siblings were determined. The distribution of mismatched haplotypes between RSV patients and unaffected siblings was not different from that expected under the null hypothesis of no linkage. (H_0: P_0=1/4 p_1=1/2 p_2=1/4. Results are given in Tables VII and VIII.

1.6.2.2 RSV patients compared to "affected" siblings in multiple case families

Seven families were available where a second sibling had upper respiratory tract disease. These families were analyzed separately. RSV infection cannot be verified in these "affected" siblings however could represent a susceptibility to upper respiratory tract disease in general.

The number of mismatched haplotypes were determined between "affected" sibling pairs are given in Table IX. The distribution of mismatched haplotypes was not significantly different from that expected under the null hypothesis of no linkage. However, a significant distortion (p 0.01) in the inheritance of the maternal haplotype was observed (Table X). Such distorted HLA haplotype inheritance has been reported in other disease families (23).

2. LEISHMANIA STUDY

2.1 Blood was shipped on 24 individuals (patients and family members) from the Dominican Republic to Washington, D.C. It was collected from the airport and the lymphocytes separated and cryopreserved. The blood sample on one patient was inadequate for typing.

2.2 Twenty-three individuals were typed for HLA-A,B,C. The method was as given in section 1.5.1. Results are given in Table XI.

2.3 The HLA data was analyzed with the clinical and skin test data. No common HLA type was observed among the patients. Genotyping was accomplished when possible by observation of segregation and haplotypes were assigned based on segregation. No evidence of linkage to HLA was suggested from the small number of families in that the distribution of mismatched haplotypes between the patients and unaffected family members was not different from that expected under the null hypothesis of no linkage.

One family was available where one patient had two HLA identical unaffected siblings. Thus the HLA genotyping data allowed further studies to be performed by the investigators at the NIH to analyze the possible role of macrophage processing of leishmania in disease pathogenesis.

3. CHRONIC GRANULOMATOUS DISEASE

Eighteen members of an extended family pedigree were typed for HLA-A,B,C. Genotyping was accomplished by observation of segregation and haplotypes were assigned. (Table XII). The two patients with confirmed disease were HLA distinct, i.e. they shared no HLA haplotype. Therefore, HLA probably does not influence the developemnt of chronic granulomatous disease.

F. PROPOSAL FOR CONTINUATION OF CONTRACT - WORK PLAN

WORK PLAN FOR CONTRACT YEAR 03 (1 OCT 1981 to 30 SEPTEMBER 1982)

A. STUDIES ON PATIENTS WITH SEVERE VASCULITIS

Most forms of clinical vasculitis are thought to be caused by immunologic phenomena. Clinically, the vasculitis syndromes may represent one of the manifestations of immune complex disease seen following many types of infectious. In some cases the vasculitis is more severe and longer lasting

than the infection itself. It is of interest to learn if certain individuals are more susceptible to these sequelae and if this susceptibility is controlled by genes in or near the HLA region.

In preliminary studies, on a small number of patients, an association between various vasculitis syndromes and HLA-DRw4 has been found.

We propose to extend and validate this observation by typing a large number of individuals with this disease as well as available family members. It is anticipated that 50-100 patients will be entered into this study during the next contract year.

B. STUDIES ON THE RECIPIENTS OF HEPATITIS B VACCINE, ANALYSIS OF NON RESPONDERS

During the past two years field trials have been underway with a vaccine for hepatitis B. The vaccine, prepared in the laboratories of Dr. John Gerin, Professor of Microbiology at Georgetown University, has been evaluated and found to be effective by NIAID investigators, with over 95% of individuals immunized seroconverting. The vaccines are now undergoing commercial efficacy studies.

During the course of the field trials it was learned that a number of individuals failed to seroconvert despite multiple immunizations with an active preparation. It is our intention to phenotype the non responders and their family members to determine if non responsiveness is associated with HLA or linked to the human MHC.

C. STUDIES ON THE STRUCTURE OF HLA-DR ANTIGENS

These studies will be undertaken in collaboration with investigators in the Laboratory of Immunogenetics, NIAID, Bethesda, Maryland.

The formal study involves the detailed analysis of HLA-A,B,C, and DR structure and function, including sequencing of the purified molecules and preliminary analysis of the biologic properties and functions of such cell surface glycoproteins. In essence we will provide charcterized serologic reagents to assist in the biochemical studies.

Under the contract guidelines, we propose to;

1. Secure, through serum donations, high titered alloantisera that recognize appropriate HLA-A,B,C and DR and to characterize the serum for fine specificity. Useful antisera will be provided to the collaborative investigators.

2. We will screen test mouse or rat monoclonal antibodies against human HLA-A,B,C or DR antigens to determine if they detect polymorphic determinants coded for by genes in the HLA region. Monoclonal antibodies will be obtained from various sources either commercial, or as gifts from other investigators wishing to have their monoclonal antibodies tested. Reports on the chacterizations will be made available to the NIAID investigators as soon after screening as is reasonable.

3. We will attempt to prepare human monoclonal antibodies to human HLA-A,B,C or DR antigens by fusing our human myeloma line (HFB1) with B cells from immunized individuals. This will be accomplished by cryopreserving peripheral blood lymphocytes, provided by serum donors, restimulating the cells in culture with the appropriate antigen, and fusing the <u>in vitro</u> stimulated cells. Pilot projects to determine the parameters for <u>in vitro</u> restimulation and fusion are currently in progress.

4. When purified preparations of DR antigens have been prepared, we will attempt to block serotyping responses with the solubilized antigens. In addition the effects of such reagents on MLC (HTC) typing will be determined.

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TABLE I

HLA A,B,C Phenotype Frequencies (Caucasian)

Control vs. RSV

A Locus			B Locus			C Locus		
HLA	Control*	RSV**	HLA	Control*	RSV*	HLA	Control*	RSV**
A1	.253	.196	B7	.193	.370	Cw1	.065	.074
2	.474	.444	8	.175	.185	w2	.086	.074
3	.269	.185	13	.052	.037	w3	230	.222
11	.120	.111	14	.092	.037	w4	.196	.148
w23	.052	.000	18	.091	.037	w5	.120	.148
w24	.132	.148	27	.075	.148	w6	.144	.148
25	.042	.037	w35	.151	.074	w7	.061	NT
26	.070	.111	37	.033	.111	w8	.047	.037
28	.100	.185	w38	.066	.037			
29	.073	.111	w39	.042	.037			
w30	.048	.037	w41	.077	.074			
w31	.065	.074	w42	.007	.000			
w32	.073	.037	w44	.262	.296			
w33	.028	.037	w45	.014	.000			
w34	.005	.000	w47	.005	.006			
w36	.004	.000	w48	.013	.000			
			w49	.051	.000			
			w50	.023	.000			
			w51	.092	.037			
			w52	.029	.000			
			w53	.002	.037			
			w54	.000	.000			
			w55	.039	.074			
			w56	.012	.006			
			w57	.073	.000			
			w58	.021	.037			
			w59	.009	.000			
			w60	.119	.037			
			w61	.023	.000			
			w62	.097	.148			
			w63	.002	.037			
			BU	NT	.000			
			4C	NT	.000			

* Control N=867 Control population from 8th International Workshop data.
** RSV patient N=27
*** $p<0.01$ (uncorrected)

TABLE II

HLA A,B,C Phenotype Frequencies (Blacks)

Control vs. RSV

A Locus			B Locus			C Locus		
HLA	Control*	RSV**	HLA	Control	RSV	HLA	Control	RSV
A1	.065	.166	B7	.147	.278	Cw1	.011	.000
2	.296	.277	8	.044	.166	w2	.179	.194
3	.177	.222	13	.022	.000	w3	.174	.278
11	.016	.000	14	.076	.083	w4	.304	.250
w23	.242	.222	18	.082	.166	w5	.054	.028
w24	.032	.028	27	.027	.000	w6	.136	.083
25	.016	.028	w35	.141	.083	w7	.076	NT
26	.065	.083	37	.011	.000	w8	.011	.083
28	.118	.222	w38	.000	.000			
29	.124	.028	w39	.011	.028			
w30	.210	.194	w41	.016	.028			
w31	.075	.000	w42	.125	.083			
w32	.027	.000	w44	.141	.056			
w33	.097	.194	w45	.092	.138			
w34	.145	.083	w47	.000	.000			
w36	.027	.056	w48	.022	.000			
19v	NT	.056	w49	.071	.000			
			w50	.022	.000			
			w51	.033	.000			
			w52	.016	.028			
			w53	.169	.222			
			w54	.000	.000			
			w55	.011	.000			
			w56	.000	.000			
			w57	.076	.056			
			w58	.147	.111			
			w59	.020	.000			
			w60	.038	.111			
			w61	.016	.000			
			w62	.016	.028			
			w63	.005	.056			
			BU	NT	.111			
			4cv	NT	.083			

* Control N=184. Control population from 8th International Workshop data.
** RSV patient N=36.
*** $p<0.01$ (uncorrected)

TABLE III

HLA DR Phenotype Frequencies (Caucasian)

HLA	Control*	RSV**
DR1	.204	.26
2	.263	.26
3	.227	.22
4	.280	.26
5	.188	.19
w6.1	.073	.04
w6.3		.15
7	.228	.22
w8	.059	.07
w9	.025	.04

* Control N = Control population from 8th International Workshop data

** RSV Patient population N = 27

*** $p < 0.01$ (uncorrected)

Table IV

HLA-DR Phenotype Frequencies (Black)

HLA	Control*	RSV**
DR1	.137	.17
2	.339	.23
3	.28	.37
4	.08	.06
5	.29	.23
6.1	.089	.14
6.3		.07
7	.22	.11
w8	.125	.11
w9	.048	.06

* Control N = Control population from 8th International Workshop data

** RSV patient population N = 36

*** $p < 0.01$ (uncorrected)

TABLE V

RSV PATIENT AND UNAFFECTED SIBLINGS GENOTYPES

(Parents a/b x c/d)

Family Member	Patient's Genotype	Unaffected Siblings Genotypes			
1	b/c	a/c	b/c	b/c	
2	a/c	a/c			
4	a/c	a/c			
5	a/c	b/c	b/d	a/c	
6	a/c	a/d	a/d	a/d	b/c
7	a/c	b/d	a/d		
9	a/d	a/d			
11	a/c	a/c			
12	b/d	a/c			
13	b/d	a/d	b/c		
14	a/c	a/d			
16	a/c	a/d			
17	b/d	a/d			
18	a/c	b/d	b/d		
19	b/c	b/c			
20	a/c	b/c			
21	a/d	a/c			
23	a/c	b/c	b/c		
26	a/d	b/d	b/d		
27	a/d	a/c			
29	a/c	b/c			
30	a/d	b/c	b/c		
31	a/c	b/c	b/c		
32	a/c	a/c			
33	a/c	b/c			
34	a/c	a/d			
37	b/c	b/c	b/c		
39	b/c	b/c			
40	a/c	a/d	b/d		
41	a/c	b/c	a/c	a/d	b/c
42	b/c	b/c			
43	b/c	a/c			
44	a/c	b/d	b/d	a/d	
45	a/c	b/c	a/c		
47	a/d	b/c	a/c		
51	b/c	a/c	a/d		
52	a/d	a/d	a/c	b/c	
54	a/c	a/c	a/d	b/d	
56	b/c	a/d	a/c	b/c	a/c
57	b/c	b/c	b/d		
58	a/c	b/d			
59	a/c	a/c	a/d	b/c	
60	b/d	b/c			
61	a/c	a/d			
62	a/d	a/c			

Families not analyzed: 15, 22, 48: 1/2 siblings 28: sibling "affected"
8, 36, 46: Recombinants 53, 64: both parents not typed 49: patient not typed

TABLE VI

RSV PATIENT AND UNAFFECTED SIBLINGS GENOTYPES

(PARENTS a/? x c/d)

Family Number	Patients Genotypes	Unaffected Siblings Genotypes					
3	a/c	a/c					
10	a/d	a/c	a/c	a/c	a/c	a/d	a/d
24	a/c						
25	a/c	a/c	a/d				
35	a/c	a/d	a/c				
38	a/c	a/d	a/c				
50	a/d	a/c					
63	a/d	a/c					

TABLE VII

UNAFFECTED SIBLINGS VS RSV PATIENTS (a/b x c/d FAMILIES)

# Mismatched Haplotypes	Source of Mismatched Haplotype	1st Sibling (N=45)	Remaining (N=34)	All Siblings
2	Fa + Mo	20%	21%	20%
1	Fa	29%	24%	28%
		51%	61%	56%
1	Mo	22%	35%	28%
0	-	29%	18%	24%

$H_0 : p_0 = p\,♂ = p\,♀ = p_2 = 1/4$

All siblings: $\chi^2 = 1.25$ $p > 0.7$

1st sibling: $\chi^2 = 1.13$ $p > 0.7$

$H_0 : p_0 = 1/4 \quad p_1 = 1/2 \quad p_2 = 1/4$

All siblings: $\chi^2 = 0.73$ $p > 0.5$

1st sibling: $\chi^2 = 1.25$ $p > 0.5$

Table VIII

UNAFFECTED SIBLINGS VS RSV PATIENTS (a/? x c/d FAMILIES)

# Mismatched Haplotypes	Source of Mismatched Haplotype	1st Sibling (N=7)	Remaining (N=8)	All Siblings
1	Mo	71%	50%	40%
0	-	29%	50%	40%

$H_0 : p_1 = p_0 = 1/2$

All siblings: $\chi^2_2 = 0.60$ $p > 0.3$

1st sibling: $\chi^2 = 1.29$ $p > 0.2$

TABLE IX

AFFECTED SIBLINGS OF RSV PATIENTS

Family Number	Patient Genotype	Affected Sibling Genotype	# Mismatched Haplotypes
5	a/c A28,Bw44,Cw- / Aw23,B7,Cw7	a/c A28,Bw44,Cw- / Aw23,B7,Cw7	0
28	b/c A2,Bw60?,Cw2 / A28,Bw60,Cw3	a/c Aw30,B7,Cw- / A28,Bw60,Cw3	1 (Father)
37	b/c Aw24,B7,Cw- / A2,B18,Cw-	b/c Aw24,B7,Cw- / A2,B18,Cw-	0
41	a/c Aw23,Bw35,Cw4 / A3,B7,Cw-	b/c Aw23,Bw58,Cw6 / A3,B7,Cw-	1 (Father)
55	a/d A28,B7,Cw- / A2,B27,Cw1	a/d A28,B7,Cw- / A2,B27,Cw1	0
61	a/c A2,B7,Cw- / Aw30,Bw51,Cw-	b/c A26,Bw55,Cw3 / Aw30,Bw51,Cw-	1 (Father)
46	a/c Aw24,Bw44,Cw- / A11,Bw62,Cw3	a/c Aw24,Bw44,Cw- / A11,Bw62,Cw3	0

Severe RSV disease which requires hospitalization occurs approximately 30% more frequently in males than in females.

TABLE X

MATERNAL TRANSMISSION OF HLA HAPLOTYPE

UNAFFECTED SIBLINGS OF RSV FAMILIES WITH 1 PATIENT

# Mismatched	1st Sib (N=45)	Remaining (34)	All Siblings (79)
1 from Mother	49%	47%	48%
0 from Mother	51%	53%	52%

FAMILIES WITH 2 RSV PATIENTS

# Mismatched	+ Siblings (N=6)	- Siblings (N=10)	All Siblings (N=16)
1 from Mother	0%	30%	18%
0 from Mother	100%	70%	72%

H_0 $P_0=P_1=1/2$

+ Siblings only $p<0.05$
All Siblings $p<0.01$

$\chi^2=6.5$

1 from Father	50%	40%	44%
0 from Father	50%	60%	56%

TABLE XII

SANTA DOMINGO STUDY

HLA Typing

I.D. Number		HLA Genotype/Phenotype	Comments
#104		A2,Bw45,Cw- (Bw6) / Aw30,B8,(Cw7?) (Bw6)	brother to patient typed previously
#105		cannot type	son of patient typed previously
#106		A2,Bw51,Cw- (Bw4) / A28,Bw53,Cw4 (Bw4)	patient
#201		A2,A28; B18,(B27?); Cw2,Cw5; Bw4,w6	control
#202		Aw23,Aw32; B40(SV?); Cw2 (Bw4,w6)	control
#203		Aw23,A29; Bw45,SV?; Cw2,Cw6 (Bw6)	control
#204	c d	A19V,B18,Cw2 (Bw6) / Aw23,Bw53,Cw6 (Bw4)	Mother of patient typed previously
#205		A19V,B18,Cw2 (Bw6) / A26,Bw52,Cw- (Bw4)	brother of patient typed previously
#301	a b	A3,Bw35,Cw4 (Bw6) / Aw30,Bw42,Cw- (Bw6)	Father of #305
#302	c d	A3,Bw52,Cw- (Bw4) / Aw24,Bw35,Cw4 (Bw6)	Mother of #305
#305	a d	A3,Bw35,Cw4 (Bw6) / Aw24,Bw35,Cw4 (Bw6)	patient
#303	a b	A1,Bw52,Cw- (Bw4) / A26,Bw44,Cw5 (Bw4)	Father of #304
#304	a c	A1,Bw52,Cw- (Bw4) / A28,Bw-,Cw3 (Bw6)	patient

TABLE XI (Continued)

#401		A26,Aw30; Bw44,Bw42; (Cw2?),Cw4 (Bw4,w6)	
#402		Aw30,Aw33; B14,B7; Cw8,(Cw7?) (Bw6)	
#403		Aw30; Bw35,Bw42; (?Cw2,Cw6) (Bw6)	extended family of patient typed previously
#404		A2,Aw24; Bw44,B7,Cw8 (Bw4,w6)	
#405		A2,A28; Bw53,Bw44; Cw4,Cw5 (Bw4)	
#406		A2,A28; Bw53,Bw44; Cw4,Cw5 (Bw4)	
#501	a c	Aw33,Bw53,Cw4 (Bw4) A1,Bw62 (Cw7?) (Bw4)	sibling of #504
#502	a b	Aw33,Bw53,Cw4 (Bw4) A29,Bw49,Cw- (Bw4)	Mother of #504
#503	c d	A1,Bw62 (Cw7?) (Bw4) A28,Bw55,Cw1 (Bw6)	Father of #504
#504	a d	Aw33,Bw53,Cw4 (Bw4) A28,Bw55,Cw1 (Bw6)	Patient
#505		A3,A11; Bw35,B7; Cw4 (?Cw7); (Bw6)	Patient

Heeke Pedigree